structural diversity that can be employed for any single molecular target, it is clear that even the process of finding an initial chemical lead for a drug discovery program can be challenging.

Fortunately, a number of tools and methods have been developed to address the simple and yet very complex question of identifying a molecular starting point for a drug discovery program. Essentially, there are two general methods utilized in modern drug discovery programs, physical high throughput screening (HTS) methods[79] and virtual high throughput screening methods.[80] There is some degree of overlap between the two categories, and the use of one set of tools does not preclude the use of the other. In point of fact, they are often employed in tandem in order to improve the likelihood of success. Physical high throughput screening approaches depend on the ability to screen large compound libraries containing hundreds, thousands, or even millions of samples. Large libraries are often designed to be chemically diverse in order to cover as much of the "drug-like" chemical space as possible, but focused libraries designed to target specific types of biological targets (e.g., kinases, phosphatases) have also been employed. Physical samples for screening are available from commercial sources (e.g., Maybridge, Enamine, Aldrich, etc.), and pharmaceutical companies generally maintain an internal compound collection of proprietary compounds.

Physical HTS techniques also require sophisticated, fully automated systems capable of manipulating reagents and 96-, 384-, or even 1496-well microtiter plates, as well as reagent distribution, data acquisition, and waste disposal for thousands of samples per hour. Automated data analysis is also required in order to handle the volume of information generated in a typical high throughput screening run.

There are some key points that one must consider in evaluating the data provided by an HTS screen. First and foremost is the possibility of false positives and false negative results. In physical screening methods, the sheer number of manipulations involved leaves open the possibility that an error may occur during some facet of reagent handling (such as a clogged pipette tip). There is also the possibility that the screening sample may have degraded over time, creating "ghost samples" within the chemical library (in other words, a sample whose structure no longer matches the material originally entered into the library). In order to ensure that programs are directed towards authentically active compounds, the chemical integrity of "hit" samples is generally assessed using High-performance liquid chromatography/Mass spectroscopy (HPLC/MS) methods. In addition, biological screening is often repeated with the "hit" compounds in order to validate the HTS results.

As an alternative to physical HTS methods, it is also possible to perform virtual high throughput screening (also referred to as *in silico* screening). In this scenario, advanced molecular modeling techniques are combined with virtual chemical libraries (data files containing detailed structural information on millions of compounds) and structural data on the biological target